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Applicants: Jon Owen CURWEN and Stephen Robert WEDGE

U.S. Application Serial No. 10/555,389 **Filed: November 3, 2005**
arising from International Patent Application WO 2004/098604

Title : Therapeutic Agents Comprising an Anti-Angiogenic Agent in Combination with an Src Inhibitor and their Therapeutic Use

Examiner : Christopher R. Stone **Group Art Unit 1614**

DECLARATION

PAUL ELVIN, of Alderley Park, Macclesfield, Cheshire GB - SK10 4TG declares:-

1. That he holds the degrees of B.Tech. and Ph.D. in Biochemistry from Brunel University, Middlesex, GB - UB8 3PH. That he is a Member of the British Association for Cancer Research; British Society for Cell Biology; and the American Association of Cancer Research . That he is a Bioscientist in the Cancer and Infection Research Department of AstraZeneca UK Limited, a business of AstraZeneca PLC.

2. That he and bioscientists working under his direction have been concerned with research into the disease of cancer for the last 28 years.

3. That he and bioscientists working under his direction have tested chemical compounds for anti-tumour activity by the following test procedure:-

Test : Inhibition of human CaLu-6 lung cancer Xenograft Tumours in Athymic Nude Mice

The test measured the ability of a combination product of the invention to inhibit the growth of human CaLu-6 lung cancer cells (ATCC Cat. No. HTB-56) grown as xenograft tumours in athymic nude mice.

CaLu-6 tumour xenografts were established in the flank of female athymic Swiss nu/nu mice, by subcutaneous injection of $1x10^6$ CaLu-6 cells/mouse in 100 µl of a 50% (v/v)

solution of Matrigel (Beckton Dickinson Catalogue No. 40234) in serum free culture medium (Eagle's Minimum Essential Medium, Gibco Catalogue No 21090-022). Ten days after cellular implant, mice were allocated to groups of 8-10 animals having comparable group mean tumour volumes. Treatment was commenced on day 10 with either a single agent or agents in combination being administered orally once daily for a minimum of 21 days, usually for about 28 days. Each single agent or combination product was prepared as a ball-milled suspension in 1% polysorbate-80 vehicle and dosed at 0.1ml/10g body weight at the appropriate dose. Control animals received compound diluent only. Tumours were measured twice weekly. Tumours were measured using vernier calipers and volumes were calculated using the formula

$$(l \times w) \times \sqrt{(l \times w)} \times (\pi/6)$$

where l is the longest diameter and w the diameter perpendicular to the longest. The level of growth inhibition was calculated by comparison of the geometric mean tumour volume of the control group versus the treatment group using a Student's T test.

The following combinations of VEGF receptor tyrosine kinase inhibitors and Src kinase inhibitors were tested in the CaLu-6 tumour xenograft model :-

(i) the VEGF receptor tyrosine kinase inhibitor VTK-1 which is the compound 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-piperidinopropoxy)quinazoline,

and the Src kinase inhibitor now known as AZD0530 which is the compound 4-(6-chloro-2,3-methylenedioxyanilino)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-tetrahydropyran-4-yloxyquinazoline;

(ii) the VEGF receptor tyrosine kinase inhibitor now known as ZD6474 which is the compound 4-(4-bromo-2-fluoroanilino)-6-methoxy-7-(1-methylpiperidin-4-ylmethoxy)quinazoline

and the Src kinase inhibitor AZD0530; and

(iii) the VEGF receptor tyrosine kinase inhibitor now known as AZD2171 which is the compound 4-(4-fluoro-2-methylindol-5-yloxy)-6-methoxy-7-(3-(pyrrolidin-1-ylpropoxy)quinazoline

and the Src kinase inhibitor AZD0530.

The resultant data are attached below by way of the graphs of Figures 4, 5 and 6.

4. Observations

That from the data provided in Figures 4, 5 and 6, he considers that the conclusions below can reasonably be drawn.

The data in Figures 4, 5 and 6 show that, in each case, each VEGF receptor tyrosine kinase inhibitor and the Src kinase inhibitor AZD0530 demonstrated activity in the CaLu-6 lung cancer model when dosed alone, but that each combination of a VEGF receptor tyrosine kinase inhibitor with that Src kinase inhibitor demonstrated an improved anti-tumour effect.

Figure 4

Figure 4 shows the results of an experiment involving the VEGF receptor tyrosine kinase inhibitor VTK-1 and the Src kinase inhibitor AZD0530. Treated animals received either VTK-1 at 3 mg per kg p.o. or AZD0530 at 50 mg per kg p.o., each dosed as single agents. In order to deliver a combined treatment, appropriate weights of the two agents were ball-milled together in 1% polysorbate-80 to provide suspensions such that a single p.o. dose delivered VTK-1 at 3 mg per kg plus AZD0530 at 50 mg per kg. Treatment was continued for 28 days and control animals received 1% polysorbate-80 vehicle only, 0.1ml/10g p.o. once daily for 28 days.

Both VTK-1 and AZD0530 alone inhibited Calu-6 tumour growth compared to the growth of tumours in vehicle treated controls. Treatment with VTK-1 alone resulted in a moderate inhibition of Calu-6 tumour growth of about 39% compared to control animals after 21 days treatment. AZD0530 alone inhibited tumour growth by about 51% after 21 days treatment. In contrast, after 21 days treatment combination of VTK-1 at 3 mg per kg with AZD0530 at 50 mg per kg inhibited tumour growth by about 70% compared to controls. Combination of VTK-1 with AZD0530 was significantly more effective when compared to VTK-1 alone ($p<0.001$). Combination treatment was similarly significantly more effective when compared to AZD0530 alone ($p<0.001$).

Conclusion : That he considers from the graphs in Figure 4 that, after 28 treatment days, the combination treatment gave a statistically significant increase in the anti-tumour effect compared to the individual treatments.

Figure 5

Figure 5 shows the results of an experiment involving the VEGF receptor tyrosine kinase inhibitor ZD6474 and the Src kinase inhibitor AZD0530. Treated animals received either ZD6474 at 25 mg per kg p.o. or AZD0530 at 50 mg per kg p.o., each dosed as single agents.

In order to deliver a combined treatment, appropriate weights of the two agents were ball-milled together in 1% polysorbate-80 to provide suspensions such that a single p.o. dose delivered ZD6474 at 25 mg per kg plus AZD0530 at 50 mg per kg.

Both ZD6474 and AZD0530 alone resulted in a moderate inhibition of Calu-6 tumour growth of about 50% compared to the growth of tumours in vehicle treated controls. In contrast, combination treatment of ZD6474 at 25 mg per kg with AZD0530 at 50 mg per kg provided markedly greater inhibition of tumour growth at about 79%. Combination of ZD6474 with AZD0530 was significantly more effective when compared to ZD6474 alone ($p<0.001$). Combination treatment was similarly significantly more effective when compared to AZD0530 alone ($p<0.001$).

Conclusion : That he considers from the graphs in Figure 5 that, after 24 treatment days, the combination treatment gave a statistically significant increase in the anti-tumour effect compared to the individual treatments.

Figure 6

Figure 6 shows the results of an experiment involving the VEGF receptor tyrosine kinase inhibitor AZD2171 and the Src kinase inhibitor AZD0530. Treated animals received either AZD2171 at 3 mg per kg p.o. or AZD0530 at 50 mg per kg p.o., each dosed as single agents. In order to deliver a combined treatment, appropriate weights of the two agents were ball-milled together in 1% polysorbate-80 to provide suspensions such that a single p.o. dose delivered AZD2171 at 3 mg per kg plus AZD0530 at 50 mg per kg.

Both AZD2171 and AZD0530 alone resulted in a moderate inhibition of Calu-6 tumour growth of about 55% compared to the growth of tumours in vehicle treated controls. In contrast, combination treatment of AZD2171 at 3 mg per kg with AZD0530 at 50 mg per kg provided markedly greater inhibition of tumour growth at about 76%. Combination of AZD2171 with AZD0530 was significantly more effective when compared to AZD2171 alone ($p<0.001$). Combination treatment was similarly significantly more effective when compared to AZD0530 alone ($p<0.001$).

Conclusion : That he considers from the graphs in Figure 6 that, after 24 treatment days, the combination treatment gave a statistically significant increase in the anti-tumour effect compared to the individual treatments.

5. **Declaration**

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and on belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and such wilful false statements may jeopardise the validity of Application Serial No. 10/555,389 or any patent issuing thereon.

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Figure 4

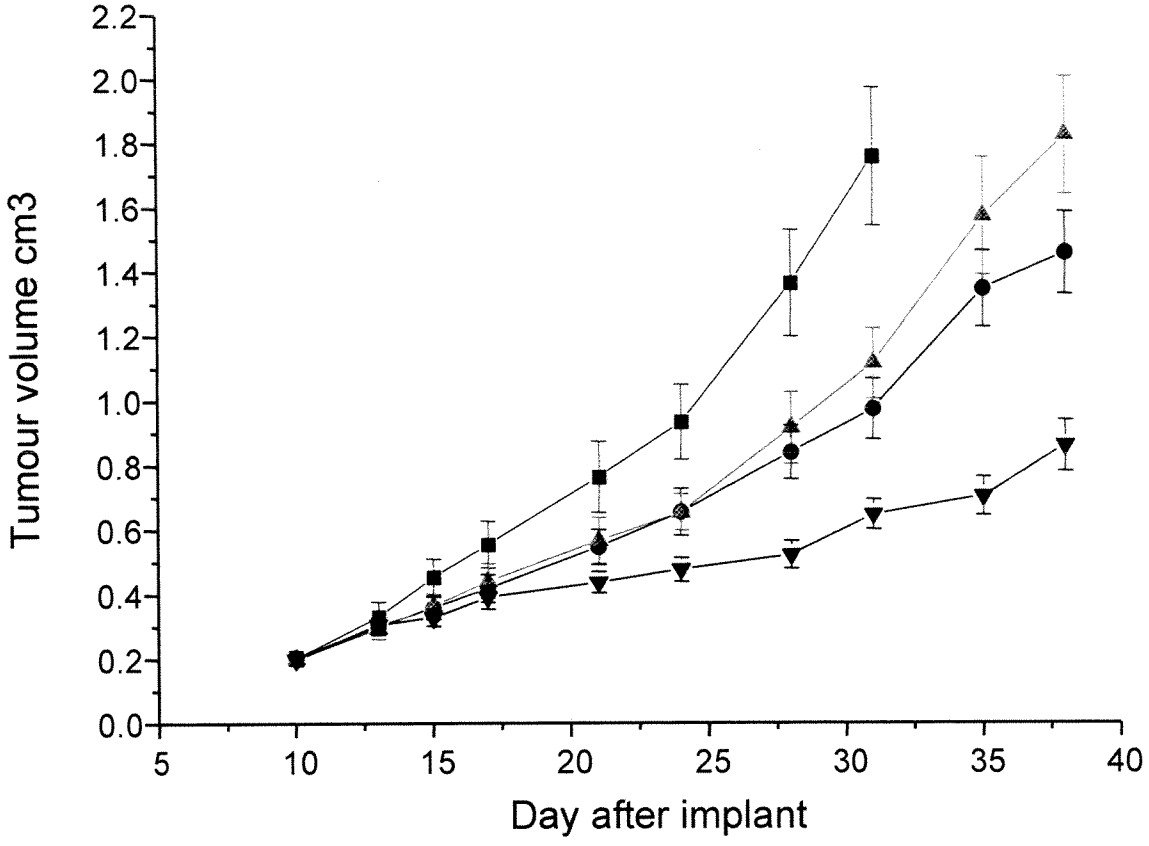

Figure 4 : Calu-6 tumour growth in control animals (■) and
response to treatment with AZD0530 alone (●) at 50 mg per kg p.o. once daily,
with VTK-1 alone (▲) at 3 mg per kg p.o. once daily, or
with a combination of AZD0530 and VTK-1 (▼) at 50 and 3 mg per kg
respectively p.o. once daily.

Figure 5

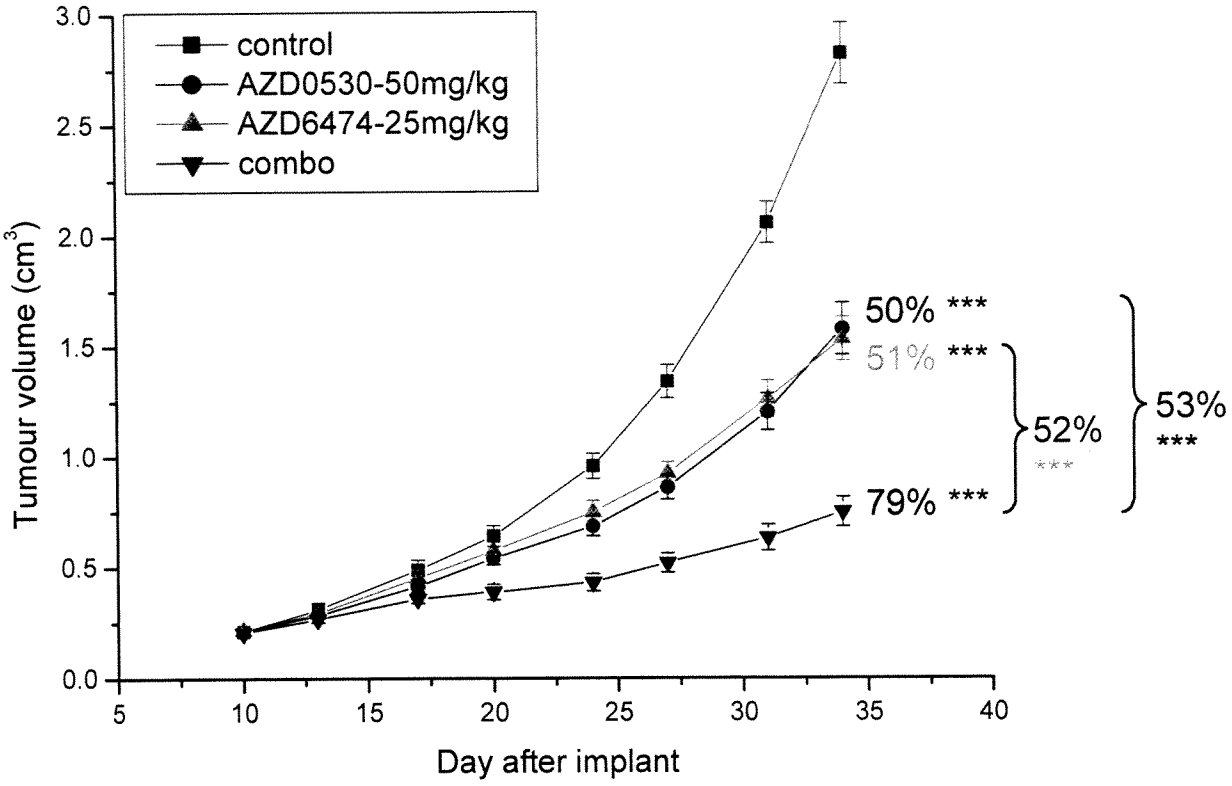

Figure 5 : Calu-6 tumour growth in control animals (■) and response to treatment with AZD0530 alone (●) at 50 mg per kg p.o. once daily, with ZD6474 alone (▲) at 25 mg per kg p.o. once daily, or with a combination of AZD0530 and ZD6474 (▼) at 50 and 25 mg per kg respectively p.o. once daily.

Significance by Student's t-test : *** means $p < 0.001$

Figure 6

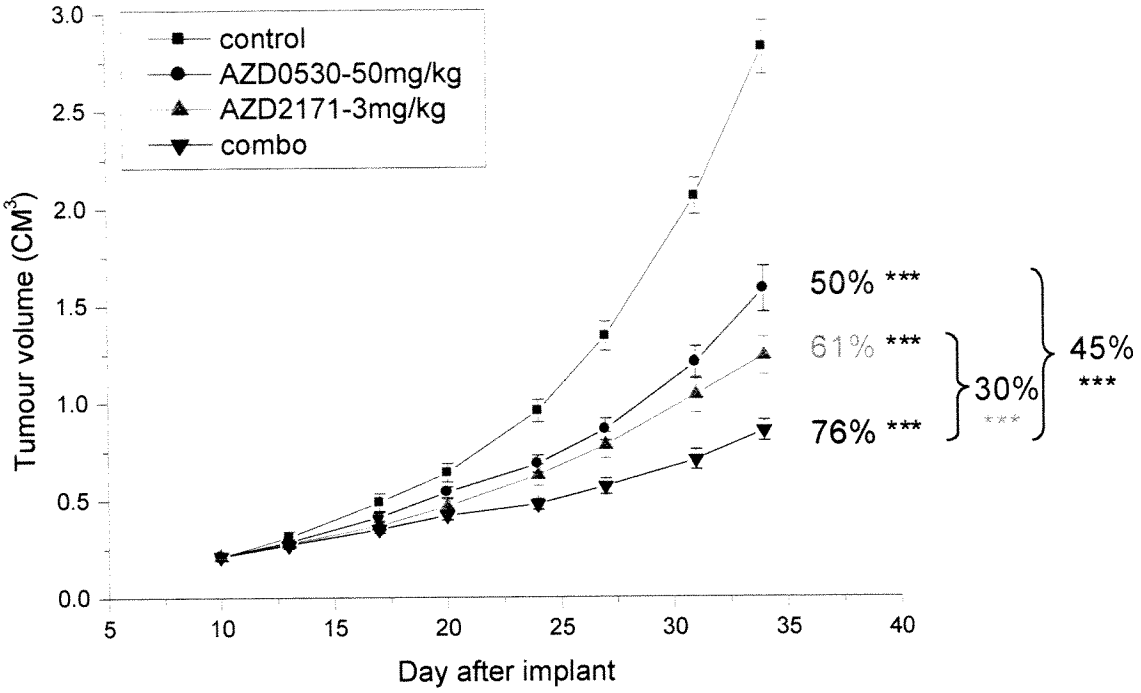

Figure 6 : Calu-6 tumour growth in control animals (■) and response to treatment with AZD0530 alone (●) at 50 mg per kg p.o. once daily, with AZD2171 alone (▲) at 3 mg per kg p.o. once daily, or with a combination of AZD0530 and AZD2171 (▼) at 50 and 3 mg per kg respectively p.o. once daily.

Significance by Student's t-test : *** means $p < 0.001$